Section I. (Amendments to the Specification)

Please amend the specification as follows:

At page 2 of the specification, please replace the paragraph beginning at line 19 with the following new replacement paragraph:

> Hyaluronic acid is a polymer of natural origin. More specifically, it is a glycosaminoglycan present in the extracellular matrix of connective tissues, such as subcutaneous tissue and cartilage, as well as in the vitreous body of the ocular globe and in the synovial fluid of articular cavities. It is a polymer ~~which has~~ for which there exist receptors, CD44 and RHAMM being predominant, which are located in the cell surface in practically all the organism's cells, with the exception of red blood cells. The interaction of hyaluronic acid with these receptors allows certain physiological processes such as mobility and cell proliferation to be regulated. Due to these properties, hyaluronic acid has therapeutic use, as it plays an important role in processes such as ~~embryo morphogenesis and development~~ morphogenesis and embryo development, cancer and inflammation. Furthermore, due to said properties, hyaluronic acid is used to promote epithelial healing. Proof of this biological activity are the numerous works that include hyaluronic acid as active biomolecule, for example, those described by Sand et al., Acta Ophthalmol. 67, 1989, 181-183, where hyaluronic acid is applied in the treatment of keratoconjuntivitis sicca and Nishida et al., Exp. Eye Res 53, 1991, 753-758, where it is applied as a wound healing agent in the cornea.

At page 3 of the specification, please replace the paragraph at line 19 with the following new replacement paragraph:

> Document WO9606622 claims the use of hyaluronic acid and derivatives, alone or in combination with another therapeutic agent, to modulate the cellular activity of those tissues and ~~receptors~~ cells which express receptors for hyaluronic acid on their surface, and thus treat or prevent inflammatory processes, fibrosis or oncogenesis.

At page 8 of the specification, please replace the paragraph at line 17 with the following new replacement paragraph:

> Document **WO9918934** relates to nanoparticles which consist of a nucleus formed from a positively- or negatively-charged polymer and a coating form from the combination of both. Ultrasounds need to be applied during the production method thereof. The particles are stabilised by the reaction thereof with a crosslinking agent (a dextran polyaldehyde, a photocrosslinking polymer or a glutamil transferase.)

At page 9 of the specification, please replace the paragraph at line 12 with the following new replacement paragraph:

> Due to the aforementioned, the present invention relates to the combination of two polymers, hyaluronic acid and chitosan, being able to substitute chitosan for other positively-charged polymers of natural origin, such as collagen and gelatine, to obtain a nanoparticulate system. Likewise, a method has been found for the preparation of nanoparticles which gives rise to the formation of same in a controlled manner and which dispenses with the use of organic solvents as well as extreme conditions. Therefore, it thus preserves the integrity of the macromolecules incorporated in the system, which is susceptible to be degraded. To achieve the formation of nanoparticles in a desired size range, it resorts to the addition of a polyanionic salt which will lead to the gelling of the positively-charged polymer, simultaneous with the ionic interaction with hyaluronic acid. It is, therefore, an ionic ~~gelling~~ gelling/interaction method which occurs in a controlled manner and will provide stability to the system, without the need to create covalent bonds between the components. These nanoparticles will have advantages with respect to other systems of greater size (microparticles, pellets, vedas, films, sponges...) with regard to their biological applications. Indeed, it is known that the interaction of a drug-release system with a biological ~~system~~ surface is highly conditioned by its size. Thus,

nanoparticles are capable of crossing epithelials and mucous membranes acting as drug transport systems, whilst microparticles do not have that capacity. The biodistribution of these systems is also highly conditioned by size. The knowledge generated in recent years in drug-release colloidal systems has allowed a clearly defined frontier to be set between the colloidal systems (less than one micron) and microparticulate systems.

At page 11 of the specification, please replace the paragraph at line 19 with the following new replacement paragraph:

According to a preferred embodiment, the hyaluronic acid salt is the sodium salt thereof. Preferably, the positively-charged polymer will be chitosan, [[it]] also being possible to use collagen or gelatine.

At page 11 of the specification, please replace the paragraph at line 23 with the following new replacement paragraph:

Also preferably, the polyanionic salt will be selected from the phosphates group, taking the sodium triphosphate as model due to the high number of negative charges ~~the~~ its structure has.

At page 12 of the specification, please replace the paragraph at line 14 with the following new replacement paragraph:

In accordance with this additional stage, the present invention also relates to hyaluronic acid nanoparticles and a positive polymer in ~~lyophil form~~ lyophilized-form and a pharmaceutical or cosmetic composition which includes them, as well as at least one pharmaceutically or cosmetically acceptable excipient.

At page 12 of the specification, please replace the paragraph at line 20 with the following new replacement paragraph:

The nanoparticles disclosed herein have suitable stability both in suspension and in ~~lyophil form~~ lyophilized form, for which reason they can be stored for long periods of time. Furthermore, their stability has also been studied in certain biological fluids which guarantee that they will remain in nanoparticulate form after their administration to human or animal organisms.

At page 13 of the specification, please replace the paragraph beginning at line 1 with the following new replacement paragraph:

The active ingredient to be incorporated in the nanoparticles comprising hyaluronic acid will have suitable pharmacotherapeutical properties for the therapeutic application for which the formulation is intended. The effect of the incorporated macromolecules ~~incorporated~~ on the human or animal organism will have the object of curing, minimising or preventing an illness, after being administered.

At page 15 of the specification, please replace the paragraph beginning at line 1 with the following new replacement paragraph:

During the exposition of the following examples, a series of abbreviations will be used:
HANa: Hyaluronic Acid Sodium Salt
CS: Chitosan
TPP: Sodium ~~triphosphate~~ tripolyphosphate
FITC-BSA: Albumin marked with fluoresceine
CsA: Cyclosporin A
SLF: Simulated lacrimal fluid

At page 15 of the specification, please replace the paragraph beginning at line 10 with the following new replacement paragraph:

Hyaluronic acid nanoparticles in the form of sodium salt, chitosan as cationic polymer and sodium ~~triphosphate~~ tripolyphosphate as crosslinking agent, were prepared according to the previously described method. The hyaluronate and sodium ~~triphosphate~~ tripolyphosphate solution were added to the chitosan solution,

with magnetic stirring, which is maintained for half an hour, permitting the complete evolution of the system towards a stable nanoparticulate form. Once prepared, their mean diameter is measured, as well as their surface electric charge (zeta potential) and the production yield is calculated (which is expressed in percentage and takes into account the weight of the nanoparticles with respect to the weight of the incorporated polymers). Table 1 and Figures 1, 2 and 3 show the values which are taken as said parameters in accordance with the proportion of HA-Na, Cs and TPP.

At page 16 of the specification, please replace Table 1 with the following new replacement Table 1:

HA-Na/CS/TPP (w/w)	Mean diameter (nm)	-Potential (+mV)	Production yield
1/1/0.05	769±36	+36.09±0.99	43±0.5
1/1/0.1	696±129	+34.50±0.28	53±3
1/1/0.15	585±9	+32.90±0.42	64±3
1/1/0.2	782±36	+31.90±0.42	75±1
1/2/0.1	550±42	+34.95±1.14	38±4
1/2/0.2	509±48	+32.63±0.68	55±1
1/2/0.3	584±26	+32.60±0.52	87±8
1/2/0.4	576±100	+31.66±0.78	82±14
1/3/0.15	539±52	+38.16±0.57	19±2
1/3/0.33	442±53	+32.63±0.71	40±3
1/3/0.5	420±16	+36.76±0.84	58±5
1/3/0.66	379±34	+35.33±1.93	72±3
1/10/0.5	634±55	+46.11±1.69	6±2
1/10/1	396±39	+44.78±1.55	15±1
1/10/1.5	312±29	+42.05±1.42	21±6
1/10/2	290±24	+41.59±2.22	34±12

At page 16 of the specification, please replace the paragraph beginning at line 4 with the following new replacement paragraph:

Hyaluronic acid nanoparticles in the form of sodium salt, chitosan as cationic

polymer and sodium ~~triphosphate~~ tripolyphosphate as crosslinking agent, were prepared according to the previously described method. A hydrophilic molecule was then incorporated in its composition, selecting FITC-BSA for said purpose. It is a negatively-charged macromolecule in both solutions due to the pH thereof (3 in the case of the chitosan solution and between 8-8.5 in the case of the hyaluronate and tripolyphosphate solutions), for which reason it was incorporated together with the hyaluronic acid to avoid the appearance of interferences in particle formation.

At page 17 of the specification, please replace the paragraph beginning at line 11 with the following new replacement paragraph:

Hyaluronic acid nanoparticles in the form of sodium salt, chitosan as cationic polymer and sodium ~~triphosphate~~ tripolyphosphate as crosslinking agent, were prepared according to the previously described method. A hydrophobic molecule was then incorporated in its composition, taking for this the polypeptide cyclosporin A, an immunomodulator agent which is practically insoluble in water, especially at moderate temperatures. The preparation method is the one already disclosed in the present invention, with one modification, since the macromolecule is previously dissolved in a 50%(V/V) acetronitrile/water solution, with a concentration of 10mg/mL. Then, a small volume of this solution, approximately 200 μL is added to the chitosan solution, and immediately afterwards the solution which contains the hyaluronic acid salt and the crosslinking agent is added. The drug encapsulation has the form of nanocrystals, which justifies the addition process of the second solution being fast, avoiding the macromolecule from precipitating and facilitating the incorporation of nanoparticles.

At page 18 of the specification, please replace the paragraph beginning at line 17 with the following new replacement paragraph:

Hyaluronic acid nanoparticles in the form of sodium salt, chitosan as cationic polymer and sodium ~~triphosphate~~ tripolyphosphate as crosslinking agent, were prepared according to the previously described method. Particle size and surface charge measurements were made, during one month, with the aim of obtaining information on the system evolution with time. For this, different formulations were selected with different quantities of hyaluronic acid. The theoretical

HANa/CS/TPP proportions were 1/2/0.4(♦), 1/2.5/0.25(●), 1/3/0.5(■), 1/3/0.66 (—) and 1/10/1.5 (▲). The results presented in figures 4 and 5 showed the little variability of the parameters, size and zeta potential, during the storage.

At page 19 of the specification, please replace the paragraph beginning at line 2 with the following new replacement paragraph:

Nanoparticles of hyaluronic acid, chitosan and TPP were prepared according to the present invention. A hydrophobic molecule, CsA, was incorporated in the form described in example 3. Then, the diameter of the nanoparticles was measured throughout one week to check he system stability with time. It has also been verified that the drug is incorporated in the particles and not precipitated in the form of nanocrystals, as no type of crystalline growth was observed. The theoretical charge of CsA was set at a percentage of 25% with respect to the nanoparticle mass. The proportions of the particle-forming polymers and the crosslinking agent, HANa/CS/TPP, were 1/2/[[04]]0.4 (♦) and 1/3/0.5(■).

At page 19 of the specification, please replace the paragraph beginning at line 17 with the following new replacement paragraph:

Hyaluronic acid nanoparticles in the form of sodium salt, chitosan as cationic polymer and sodium ~~triphosphate~~ tripolyphosphate as crosslinking agent, were prepared according to the previously described method. A proportion of HANa/CS/TPP of 1/2/0.4 was used, and the effect that the type of cryoprotective agent used in the lyophilisation process has on the size was checked on these particles. The influence on the nanoparticle concentration in the suspension to lyophilise was also evaluated. After preliminary assays, two sugars, glucose and trehalose, were selected as cryoprotective agents and their concentration was kept constant, setting it at 5% (w/V).

At page 20 of the specification, please replace the paragraph beginning at line 2 with the following new replacement paragraph:

Hyaluronic acid nanoparticles in the form of sodium salt, chitosan as cationic polymer and sodium ~~triphosphate~~ tripolyphosphate as crosslinking agent, were

prepared according to the previously described method. The formulation developed was that with composition HANa/CS/TPP: 1/2/0.4 and it was lyophilised for 48 hours using 5% glucose as cryoprotective agent. Then, a mucoadhesion study was performed, using SLF and a 4% mucin solution for this.

At page 20 of the specification, please replace the paragraph beginning at line 10 with the following new replacement paragraph:

Hyaluronic acid is a polymer with a viscoelastic behaviour in gel-form. In the case of colloidal suspensions, the rheological behaviour is more complex; the viscosity is highly influenced by the particle's surface properties.